510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041357

B. Purpose for Submission:
New device

C. Analyte:
Anti-Gliadin IgG antibodies

D. Type of Test:
Qualitative and semi-quantitative, EIA

E. Applicant:
Sweden Diagnostics (Germany) GmbH
Pharmacia Diagnostics AB

F. Proprietary and Established Names:
Varelisa® Gliadin IgG Antibodies

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5750, Radioallergosorbent (RAST) immunological test system
2. Classification:
   Class II
3. Product Code:
   MST, Antibodies, Gliadin
4. Panel:
   Immunology (82)

H. Intended Use:
The Varelisa® Gliadin IgG Antibodies EIA kit is designed for the semiquantitative and qualitative determination of gliadin (IgG) antibodies in serum or plasma to aid in the diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis.

1. Indication(s) for use:
   Same as Intended Use.
2. Special condition for use statement(s):
   The device is for prescription use only.
3. Special instrument Requirements:
   Microplate reader capable of measuring OD at 450 nm and with a reference filter at 620 nm.

I. Device Description:
The assay kit consists of (1) 12 or 6 gliadin antigen coated microplate strips, (2) horseradish peroxidase conjugated anti-human IgG, (3) TMB substrate, (4) ready-to-use 6 level calibrators (gliadin IgG antibody concentrations of 0, 3, 7, 16, 40 and 100 U/mL), (5) ready-to-use positive control, (6) ready-to-use negative control, (7) wash buffer concentrate (20x), (8) sample diluent concentrate (5x) and (9) stop solution. Calibrators, positive and negative controls are diluted human sera.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA QUANTA Lite™ IgG Gliadin ELISA
2. Predicate K number(s):
   k984985
3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Intended Use. for the semi-quantitative and qualitative determination of gliadin (IgG) antibodies in serum or plasma to aid in the diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis	For the semi-quantitative detection of Gliadin IgG antibodies in human serum. Detection of these antibodies is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis
Assay type – ELISA	Same
Analyte – Anti-gliadin IgG antibodies	Same
Capture antigens – purified gliadin antigen (wheat)	Purified gliadin antigen (unknown source)
Conjugate - Horseradish peroxidase	Same
Substrate – TMB	Same
Sample dilution – 1:101	Same
B. Differences	
Assay format – Qualitative and semi-quantitative	Semi-quantitative
Sample type – Serum and plasma	Serum
Calibrators – 6 levels	None
Controls – positive and negative	Negative, low positive and high positive controls
Cut-off values	
Semi-quantitative - Negative <11 U/mL Equivocal 11-17 U/mL Positive >17 U/mL	Negative <20 units weak positive 20-30 units moderate to strong positive >30 units
Qualitative – Negative ratio <1.0 Equivocal ratio 1.0-1.4 Positive ratio >1.4	

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

The Varelisa® Gliadin IgG Antibodies assay is an indirect noncompetitive enzyme immunoassays. The wells of a microplate are coated with gliadin antigen. Diluted patient samples are added to the microplate wells and antibodies specific for gliadin if present will bind to the immobilized antigen. Unbound samples are washed away and an enzyme labeled second antibody (conjugate) is added to each well and bind to the antigen/antibody complex to form an enzyme labeled conjugate-antibody-antigen complex. After washing away any unbound enzyme conjugate, the chromogenic substrate is added. The enzyme labeled antigen-antibody complex converts the substrate to form a color solution. The rate of color formation is a function of the amount of conjugate complexed with the bound antibody and therefore is proportional to the concentration of the autoantibody in the patient sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three samples (low, medium, high) from a serum bank were diluted and assayed for 5 runs with 4 replicates per run. Within one day one operator performed the analyses. Calibrators and Controls were analyzed in triplicates. Target values established for this study included within-run variance <9% and between assay <9%. The specifications were met. The table below summarizes the results.

Sample ID		Run 1	Run 2	Run 3	Run 4	Run 5	Mean (U/mL)	Variance	
								Within	Between
1	Mean (U/mL)	14.0	14.7	14.1	14.3	14.0	14.2	2.1	1.6
2	Mean (U/mL)	28.1	29.8	27.5	28.6	28.7	28.6	2.5	2.7
3	Mean (U/mL)	42.2	43.8	40.5	40.9	42.4	42.0	2.6	2.8

b. Linearity/assay reportable range:

Dilution study - Three positive serum samples from a serum bank were pre-diluted to 1:101 followed by further dilution with Sample Diluent to 1:1, 2:3, 1:2, 1:4, 1:8, 1:16, and 1:32. Calibrators, Controls and each dilution were measured in triplicates. Specifications were that observed/expected percents should be within ± 20% for at least 3 successive dilutions of each tested sample. The dilutions met the criteria and were considered linear.

Recovery study – Two samples containing 3.7 U/mL and 20.9 U/mL of gliadin IgG from a serum bank were diluted to 1:101 and spiked with 1/10 volume of Calibrator points S1, S2, S3, S4, S5 and S6, i.e. 0.3, 0.7, 1.6, 4 and 10 U/mL respectively. The unspiked and spiked samples, the Calibrators and controls were measured in duplicates. Acceptance criteria were that % recoveries should be within ± 20% of the expected values. The percent recoveries ranged from 93.2% to -97.7%. Study results met the acceptance criteria.

Reportable range – 0.3 U/mL to 100 U/mL

c. Traceability (controls, calibrators, or method):

There is no recognized reference material for gliadin antibodies. Results are reported in arbitrary units

d. Detection limit (functional sensitivity):

Sample Diluent was diluted according to Directions for Use and measured 56 times on one plate. Calibrators and Controls were analyzed in quadruplicates. Analytical sensitivity was calculated as the mean of the optical densities (OD) of the Sample Diluent plus 3SD and expressed in U/mL. The discrimination value D for

differentiating the lowest calibrator point and the background was calculated using the following equation:

$$D = \frac{\eta_B - \eta_A}{\sqrt{(\sigma_B^2 - \sigma_A^2)}}$$

where A = Sample Buffer; B = Calibrator S2; $\acute{\eta}_A$, $\acute{\eta}_B$ = mean OD; σ_A, σ_B = SD.

Acceptance criteria specified that the (mean OD + 3SD) of the Sample Diluent < Calibrator point S2, detection limit ≤1 U/mL and the discrimination value D >2.0. The (mean +3SD) ODs for the Sample Diluents from the three different kits were 0.026, 0.021 and 0.022, which corresponded to a mean analytical sensitivity of 0.3 U/mL. The other criteria were also met.

e. Analytical specificity:

Interference was tested against potentially interfering substances found in blood: bilirubin, hemoglobin, chyle, and rheumatoid factor. Three samples with known gliadin IgG concentrations from a serum bank were diluted 1:101 and spiked with buffer or different amounts of interfering substances. The spiked and unspiked samples were analyzed in triplicates. The Calibrators and Controls were analyzed in duplicates. Acceptance criteria were that spiked samples should be ≤20% variation from unspiked sample. The concentrations spiked in are shown below.

Additives	Final sample concentration				
	Blank	II	III	IV	V
Bilirubin F (mg/dL)	0.0	4.6	9.1	13.7	18.2
Bilirubin C (mg/dL)	0.0	5.5	11.0	16.5	22.0
Chyle (Units)	0.0	700	1400	2100	2800
Hemoglobin (mg/dL)	0.0	122.5	245.0	367.5	490.0
RF (IU/mL)	0.0	110.0	330.0	550.0	n.a.-

Except for chyle with significantly elevated results, the other substances did not show significant interference.

Additives	Analyte Conc.	% Recovery				
		Blank	Conc. I	Conc. 2	Conc. 3	Conc. 4
Bilirubin C	19.1	0	-98.5	98.0	99.1	97.2
	26.3	0	99.3	95.5	97.2	95.8
	44.4	0	93.5	91.5	92.8	92.0
Bilirubin F	18.3	0	97.1	93.9	99.5	99.7
	24.3	0	98.4	97.3	106.4	106.4
	40.7	0	97.9	93.4	104.5	102.9
Chyle	16.4	0	117.4	123.0	122.2	123.1
	24.0	0	116.2	120.5	116.1	116.8
	38.4	0	123.8	115.3	112.6	117.0
Hemoglobin	18.0	0	104.1	96.4	96.4	94.1
	25.8	0	97.9	95.7	94.5	89.8
	41.2	0	99.4	95.7	96.9	96.1

Additives	Analyte Conc.	% Recovery				
		Blank	Conc. I	Conc. 2	Conc. 3	Conc. 4
RF	20.6	0	99.2	97.7	90.1	
	29.0	0	99.0	94.6	95.6	
	45.2	0	102.2	100.8	96.6	

Crossreactivity was assessed by testing 60 sera positive for other antibodies including ANA, SS-A, SS-B, TPO, dsDNA Cardiolipin IgG, PR3, GBM, MPO, RF, PC, LKM1, actin and HCV. Fifty sera were from an external source and 10 sera were ANA human reference sera from CDC. All samples were found negative.

f. Assay cut-off:

The semi-quantitative cut-points were determined by measuring 360 samples from apparently healthy Caucasian blood donors, equally distributed by sex and age from a serum bank. Diluted samples, Calibrators and Controls were analyzed in duplicates. Specification was that 95th percentile should be <lower limit of equivocal range. Results showed that there was no difference between gender and age. The mean and median concentration of gliadin IgG antibodies were 3.6 U/mL and 1.9 U/mL respectively. The mean+2SD was 14.2 U/mL, the mean+3SD was 19.5 U/mL and the 95 percentile was 12.5 U/mL. Based on these results, the following values were selected for negative, equivocal, and positive:

<11 U/mL = negative
11-17 U/mL = equivocal
>17 U/mL = positive

2. Comparison studies:

a. Method comparison with predicate device:

One hundred and forty-three clinically defined patient samples and 42 normal samples rom a serum bank were tested on the new device, the predicate device and IFA anti-endomysial antibody assay (EAM IIF). The patient samples consisted of 100 celiac disease (CD), 18 inflammatory bowl disease (IBD) and 17 morbus Crohn/colitis ulcerosa (Crohn/UC) and 8 unknown diagnosis (EAM IIF positive). The patient samples were analyzed in singlicates or duplicates. Calibrators and Controls were analyzed in duplicates. The age of the patient group ranged from 3 years to 71 years with a mean age (±SD) of 30.2 (±18.8) years. The age of the control group ranged from 1 year to 71 years with a mean age of 36.9 (±22.7) years. Samples from the CD cohort were EAM IIF positive whereas samples from the healthy cohort, the Crohn/UC cohort and the IBD cohort were EAM IIF negative. The concentration ranges of anti-gliadin IgG antibody for the 5 cohorts are shown in the following table.

Varelisa® Gliadin IgG (U/mL)	Cohorts				
	CD	Crohn/UC	IBD	Unknown (EMA+)	Healthy
N	100	17	18	8	42
Range	2 to >100	1.3 to 82.4	1.7 to 36.2	2.7 to >100	1.1 to >100

Results of the predicate comparison summarized below.

		INOVA QUANTA Lite™ Gliadin IgG		
		+	-	Total
Varelisa® Gliadin IgG	+	84	0	84
	-	34	48	82
	Equiv	19	0	19
	Total	137	48	185

% positive agreement = 61.3% (84/137) (95% CI: 53.1% to 69.5%)
% negative agreement = 100% (48/48)
% total agreement = 71.3% (132/185), (95% CI: 64.8% to 77.8%)

The following table is a compilation of the discrepant samples.

Sample Type	$INOVA^{Pos}/Varelisa^{Neg}$	$INOVA^{Pos}/Varelisa^{Equ}$
CD	24	6
Crohn/UC	2	2
IBD	3	7
Unk (EAM+)	0	1
Healthy	5	3
Total	34	19

b. Matrix comparison:

The device uses both serum and plasma samples. To demonstrate that the new assay gives the same results for serum, heparin plasma, citrate plasma and EDTA plasma from the same patient, 10 gliadin IgG antibody negative samples (gliadin IgG concentrations ranged from 0.9 U/mL to 2.8 U/mL) for each matrix were spiked with 10 different gliadin IgG positive sera (gliadin IgG concentrations ranged from 35.8 U/mL to 76.8 U/mL). The negative samples and the spiked samples were run in quadruplicates. Acceptance criteria for this study were that the percent deviation between serum and plasma results for positive samples should not be greater than ±20% and negative samples should be negative for both serum and plasma matrices. The data showed no difference greater than ±20% with deviations ranged from -9.4% to 11.2% for citrate, -8.3% to 13.7% for EDTA plasma and -8.0% to 6.1% for heparin. No negative sample changed from negative to positive. Thus the specifications were met.

Since all positive samples had high concentrations of anti-gliadin IgG antibody, additional data on negative and equivocal patient samples were requested. Serum and plasma samples from two additional patients (one negative with 5.3 U/mL and one equivocal with 15.7 U/mL) were analyzed and the results were within the acceptance criteria.

3. Clinical studies:

a. Clinical sensitivity:

One hundred and seventy-six clinically defined sera were analyzed using the Varelisa® Gliadin IgG Antibodies assay and the EMA IIF assay. The samples consisted of 99 CD, 18 IBD, 17 Crohn/UC and 42 normal controls. In the CD cohort, 31 samples were negative and 6 were equivocal. All these samples were EMA positive. The equivocal samples were considered negative. Based on this study, the clinical sensitivity was 62.6% (95% CI: 53.1% to 72.1%). Results are summarized below:

		Celiac disease		
		Positive	Negative	Total
Varelisa® Gliadin IgG	Positive	62	17	79
	Equivocal	6	12	18
	Negative	31	48	79
	Total	99	77	176

b. Clinical specificity:

The clinical specificity of the Varelisa® Gliadin IgG Antibodies assay was determined by the study described in 3(a). In the negative cohorts, there were 17 positive and 12 equivocal samples. Since the sponsor considered the equivocal results negative, the clinical specificity was 77.9% (95% CI: 68.6% to 87.2%).

4. Clinical cut-off:
   Same as assay cut-off.
5. Expected values/Reference range:
   Expected value in the normal population is negative. Apparently healthy asymptomatic individuals may test positive for gliadin antibodies. The frequency distribution of anti-gliadin IgG antibodies as determined by the Varelisa® Gliadin IgG Antibodies assay on 176 clinically defined samples is summarized below.

	N	#Positive	Frequency
CD	99	62	62.6%
IBD	18	3	16.7%
Crohn/UC	17	3	17.6%
Healthy	42	11	26.2%

N. Conclusion:

The submitted information in this premarket notification is complete to support a substantial equivalence decision.